



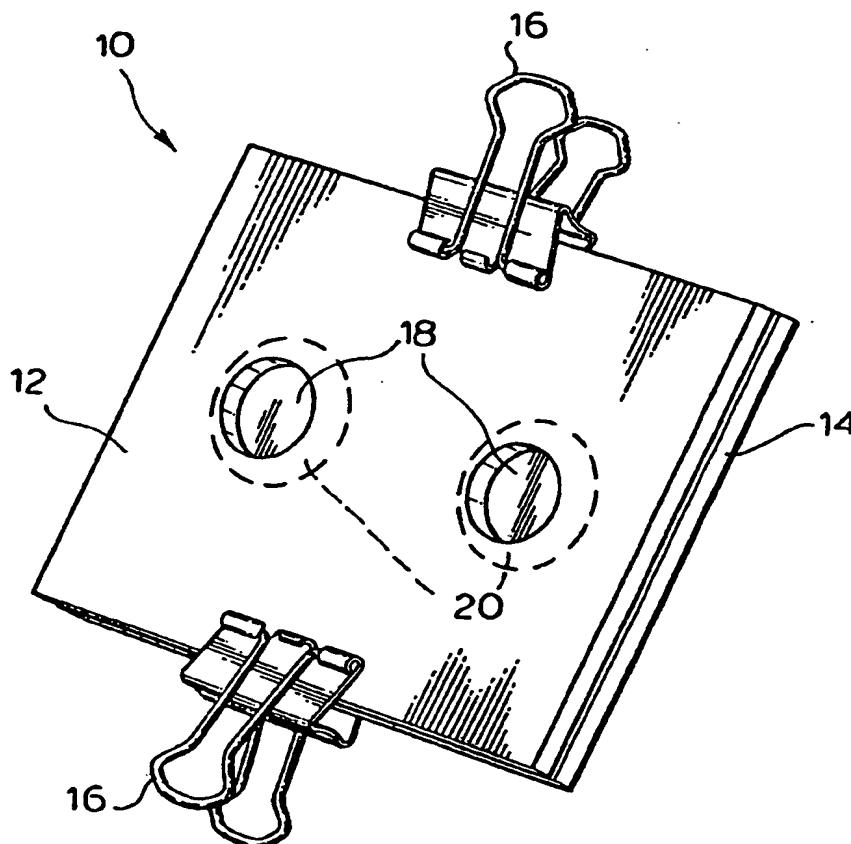
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|--|---|
| (51) International Patent Classification ⁶ : G01N 33/52, 33/574 | A1 | (11) International Publication Number: WO 95/14927 (43) International Publication Date: 1 June 1995 (01.06.95) |
| <p>(21) International Application Number: PCT/CA94/00646</p> <p>(22) International Filing Date: 23 November 1994 (23.11.94)</p> <p>(30) Priority Data: 08/158,294 29 November 1993 (29.11.93) US</p> <p>(71)(72) Applicants and Inventors: KREPINSKY, Jiri, J. [CA/CA]; 810 Srigley Street, Newmarket, Ontario L2Y 1X7 (CA). CHOCIEJ, Jacek [CA/CA]; 43 Valleywoods Road #89, Toronto, Ontario M3A 2R5 (CA). KANDEL, Gabor, P. [CA/CA]; 430 Heath Street East, Toronto, Ontario M4G 1B5 (CA). YEUNG, Ka, Sing [CA/CA]; 810 Srigley Street, Newmarket, Ontario L3Y 1X7 (CA).</p> <p>(74) Agent: SMITH, LYONS, TORRANCE, STEVENSON & MAYER; Suite 6200, Scotia Plaza, 40 King Street West, Toronto, Ontario M5H 3Z7 (CA).</p> | <p>(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> | |

(54) Title: SCREENING TEST FOR EARLY DETECTION OF COLORECTAL NEOPLASIA

(57) Abstract

A method for detecting the presence of neoplasia or cancer of the colon or rectum, which method comprises obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent and screening for neoplasia or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment. The method is rapid, simple, inexpensive and provides a screening test for colorectal cancer which does not give a high percentage of false positive and false negative results. A screening test kit is provided.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Larvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

- 1 -

SCREENING TEST FOR EARLY DETECTION OF
COLORECTAL NEOPLASIA

FIELD OF INVENTION

5 This invention relates to a simple screening test for
colorectal cancer. Specifically, a method is described
whereby a colorectal cancer marker is detected in rectal
mucus obtained by digital rectal examination. More
particularly, this marker is detected in the mucus deposited
10 on a support using Schiff's reagent.

BACKGROUND OF THE INVENTION

15 Colorectal carcinoma is the second most frequent cause
of cancer mortality in men and women, causing nearly one
third of all malignancy-related deaths in North America. It
has been estimated that ultimately as many as 6% of Canadians
and Americans will develop malignancy in the lower bowel, and
over 50% of them will die within 5 years of diagnosis.
20 Because there are no realistic prospects of significantly
improving the cure rate once the cancer has spread beyond the
bowel wall, many authorities believe that colorectal cancer
can be controlled only by preventive measures (1).

25 Primary prevention, i.e. averting the development of the
tumour by altering biological risk factors, is not yet
feasible since so little is understood of the etiology of the
disease. Alternatively, secondary preventive measures, i.e.
detection at an asymptomatic, treatable state, would be
possible should an effective screening test be available.
30 Indeed, neoplasms of the lower bowel have the characteristics
that make them a suitable candidate for the development of
a screening test. This is because i) it is a common cause
of cancer-related deaths, and ii) whereas once the stage of
true cancer is reached, leading to symptoms, the mortality
35 rate is over 50%, removal of bowel neoplasms at its earliest,
asymptomatic stage can be done by non-surgical endoscopic

- 2 -

polypectomy, without any significant risk. Moreover, it requires at least four to six years before an adenomatous polyp reaches the cancer stage, so there is ample opportunity to detect these neoplasms at their treatable stage. Recent clinical studies document a decrease in mortality from colorectal cancer screening, as predicted by these theoretical considerations. The problem to-date has been that polyps can be reliably detected only by endoscopy.

Thus, colorectal cancer satisfies each of the following three criteria of a disease considered suitable for a screening program. First, it is a relatively common condition with serious consequences. Second, curative treatment is available when detected at an early stage, i.e. snare polypectomy through a colonoscope or surgical segmental bowel resection. Third, the prevalence is sufficiently high to justify the expense of a screening program (2).

PRINCIPLES OF SCREENING

The goal of a medical screening program is to reduce morbidity and mortality by detecting a disease at a sufficiently early stage to allow curative treatment. It is not designed necessarily to diagnose a disease, but to determine which asymptomatic, apparently disease-free individuals should undergo diagnostic investigations. The ability of a screening test to distinguish those who warrant further evaluation from those who do not is expressed in epidemiological terms. The term "sensitivity" is defined as the proportion of diseased individuals who have a positive test, i.e. the proportion of true positives/all persons with the disease. "Specificity" is the proportion of disease-free subjects who have a negative test, i.e. the proportion of true negatives/all persons without the disease. The term "positive predictive value" is the proportion of positive tests due to the disease, i.e. the proportion of true positives/all positives. Almost always, sensitivity and specificity must be traded against each another. Intuitively, it appears wise to design a screening test for

- 3 -

5 a fatal disease so as to optimize sensitivity, in order to
detect as many individuals with the disease as possible. It
has been emphasized, however, that optimizing sensitivity
brings with it a risk of reducing specificity to such an
10 extent that unacceptably high costs, poor compliance, and
"flooding" of diagnostic facilities result. Moreover,
positive predictive value, which is a particularly useful
expression of the value of a screening test, is critically
dependent on specificity and on the prevalence of the disease
15 in the population screened.

It has been stressed that the effectiveness of a
screening test can be properly evaluated only by randomized
controlled trials. In the case of cancer, it is not
sufficient to demonstrate that life is prolonged when the
15 malignancy is detected by a positive screening test, compared
to when the tumour is diagnosed after the development of
symptoms. Instead, it must be shown that screened
individuals have a lower death rate from the malignancy than
similar individuals not enrolled in such a screening program.
20 Important sources of error in interpreting the results of
previous screening programs include lead time bias, length
bias, and patient selection bias. A particularly fallacious
assumption is that the predictive value of a screening test
is the same in a hospitalized population with advanced
25 disease, in which the test is usually initially tried, as it
is in a healthy population with early minimal disease, to
which the test is usually aimed.

CURRENT POPULATION SCREENING METHODS

30 Endoscopic methods, such as sigmoidoscopy or entire-
length colonoscopy, are diagnostic rather than screening
techniques. The only current method of colorectal cancer
screening in the general population is searching for occult
blood in the stool (3). Present techniques e.g. HemOccult
35 II involve smearing a sample of stool onto guaiac impregnated
paper which, after treatment with hydrogen peroxide
containing developer, exhibits blue colour if blood,

- 4 -

haemoglobin, is present. After almost two decades of experience with this methodology, it has become clear that even in expert centres, the sensitivity is less than 50% for curable neoplasms, and that the positive predictive value approximates, at best, only 40% in a clinic population. An update from the large-scale (n=97,205) University of Minnesota, Minnesota, United States, prospective trial indicates a positive predictive value for colorectal cancer of only 2.2% when HemOccult is used in asymptomatic subjects, aged 50-80, with an overall disease prevalence of 0.2% (4). Furthermore, factors such as medications, multiple dietary constituents, delays in specimen handling, variabilities in fecal hydration, and storage of assay materials commonly confound results. Analysis of one of the three randomized controlled studies assessing the value of HemOccult suggests comparable mortality rates in the screened and control populations (5). Newer methods of detecting occult blood, e.g. methods based either on porphyrin analysis [HemoQuant] or antibody specific for human haemoglobin, may improve on these results. However, three limiting problems remain unlikely to be overcome. These are that colorectal malignancies shed blood only intermittently, upper gastrointestinal tract bleeding may make the results (falsely) positive, and multiple lesions in the lower bowel, apart from colorectal neoplasms, commonly bleed. Such lesions include hemorrhoids, diverticulae, ulcers, and vascular ectasie. Compliance in unselected populations has been estimated to be less than 30%, at least partly because the technique requires patients themselves to smear their stool onto a slide or a strip, a task most people find not only distasteful, but also technically difficult. Despite this, HemOccult continues to be widely used because the American Cancer Society has recommended occult blood testing yearly for all individuals over 50 years of age, arguing that even an imperfect test will save many lives. Implicit in all arguments over the value of HemOccult is that any improvement in screening techniques for bowel malignancy would have a

- 5 -

dramatic impact on colorectal cancer mortality rates from the disease, since the screening for occult blood even in the present form leads to reducing mortality from colorectal cancer (6).

5

EXPERIMENTAL SCREENING METHODS

(i) Screening for colorectal cancer by stool DNA analysis (7).

10

15

20

25

This is based on the presence in stool of neoplastic cells shed in large numbers into the colonic lumen. In principle, a mutation which is common to neoplasms could be detected with high precision by analyzing DNA from these cells. Therefore, the existence of a detectable mutation in the colorectal tumour is a prerequisite for developing such a method of screening. Unfortunately, this technique can recognize a mutation based only on a new or altered oligonucleotide sequence, but not on a loss of its portion. Thus, neoplasia-related mutations based on deletion in genes, e.g. allele losses on chromosomes such as are commonly found in colorectal tumours, are beyond the limits of the methodology. Currently, the most common mutation is the K-ras oncogene mutation present, in about 40% of colorectal carcinomas and adenomas. Screening for K-ras gene can therefore detect, at best, only 40% of all neoplasias. This methodology is at present technically very complex and expensive.

30

(ii) Screening for the presence in colonic mucin of a cancer-related disaccharide, D-Galp(β 1-3)-D-GalpNAc(α 1,Ser/Thr), T-(Thomsen-Friedenreich) antigen, since it is widely known that T-antigen is not expressed by cells in healthy colons, whereas it is expressed by cancer (8).

35

(a) Monoclonal antibodies and lectins: It has been shown that monoclonal antibodies raised against synthetic T-antigen recognize and bind to cancer cells. Similarly, peanut agglutinin (PNA), a lectin, binds strongly to the same disaccharide, but recognizes malignancy with lesser

- 6 -

specificity. Amaranthin, a lectin from *Amaranthus caudatus*, has been reported to have better specificity for T-antigen than PNA. Neither amaranthin nor PNA bind to histological sections of normal mucosa, but both bind to mucin in the goblet cells of tumours and certain polyps, and in the transitional mucosa. The visualization of the binding utilizes fluorescently labelled antibodies and lectins (9). (b) Galactose oxidase test. T-antigen is also reported to be detectable colorimetrically after oxidation of OH-6 of galactose using galactose oxidase and visualization of the resulting aldehyde with Schiff's reagent, - United States Patent No. 4857457, issued August 15, 1989 to Shamsuddin et al. In contrast with the tests using lectins, this test is performed on mucus samples obtained by digital rectal examination and smeared onto a support. This system demonstrated a sensitivity of 74% and specificity of 50% for colorectal neoplasms, i.e. adenomatous polyps and cancer, in one study with only 1 false negative result among 59 patients with cancer. Since then a number of reports of basically the same test have appeared with sensitivity ranging from 35% to 100% and specificity ranging from 15% to 76%. Some investigators found that the test was more sensitive, but less specific, than HemOccult. The lesser specificity has been ascribed to the positivity of test in individuals with certain inflammatory condition, such as diverticulitis and ulcerative colitis (10).

REFERENCE LIST

The present specification refers to the following publications, each of which is expressly incorporated herein by reference.

PUBLICATIONS

I. Lieberman DA: Targeted colon cancer screening: A concept whose time has almost come. *Amer. J. Gastroenterol.* 1992, 87,

- 7 -

1085.

II. Eddy DM: Screening for colorectal cancer. Ann. Int. Med. 1990, 113, 373.

5 III. Rex DK, Lehman GA, Ulbright TM, Smith JJ, Pound DC, Hawes RH, Helper DJ, Wiersema MJ, Langefeld CD, Li W: Colonic neoplasia in asymptomatic persons with negative fecal occult blood tests: influence of age, gender, and family history. Am. J. Gastroenterol. 1993, 88, 825.

10 IV. Mandel JS, Bond JH, Bradley M, Snover DC, Church TR, Williams S, Watt G, Schuman LM, Ederer F, Gilbertsen V: Sensitivity, specificity, and positive predictivity of the Hemoccult test in screening for colorectal cancer. Gastroenterol. 1989, 97, 597.

15 V. Selby JV, Friedman GD, Quesenberry, Jr. CP, Weiss NS: Effect of fecal occult blood testing on mortality from colorectal cancer. Ann. Intern. Med. 1993, 118, 1.

20 VI. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F: Reducing mortality from colorectal cancer by screening for fecal occult blood. New Engl. J. Med. 1993, 328, 1365.

VII. Editorial: Screening for colorectal cancer by stool DNA analysis. Lancet 1992, 339, 1141.

25 VIII. Boland CR, Montgomery CK, Kim YS: Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation. Proc. Natl. Acad. Sci. USA 1982, 79, 2051.

30 IX. Rinderle SJ, Goldstein IJ, Matta KL, Ratcliffe RM: Isolation and characterization of Amaranthin, a lectin present in the seeds of Amaranthus caudatus, that recognizes the T- (or cryptic T) antigen. J. Biol. Chem. 1989, 264, 16123.

X. Sakamoto K, Muratani M, Ogawa T, Nagamachi Y: Evaluation of a new test for colorectal neoplasms: a prospective study of asymptomatic population. Cancer Biotherapy 1993, 8, 49.

35 XI. Robins JH, Abrams, GD, Pincock JA: The structure of schiff reagent aldehyde adduct and the mechanism of the Schiff reaction as determined by nuclear magnetic resonance

- 8 -

spectroscopy. Can. J. Chem. 1980, 58, 339.

XII. Kasten FH: The chemistry of Schiff's reagent. 1960, Int. Revs. Cytol. 10, 1.

XIII. Shamsuddin A: Diagnostic assays for colon cancer. CRC Press, Boca Raton FL, 1991.

PATENTS

1. U.S.P. 4857457, Shamsuddin et al, August 15, 1989.
2. U.S.P. 4762800, Rettig et al, August 9, 1988.
3. U.S.P. 4863854, Mattes et al, September 5, 1989.
4. U.S.P. 4962187, Pant, October 9, 1990.
5. U.S.P. 5073493, Yamashina, December 17, 1991.
6. U.S.P. 5008184, Linnane, April 16, 1991.

SUMMARY OF THE INVENTION

In contrast to the above-noted prior art, we have surprisingly discovered that mucus collected from individuals with neoplastic disease of the colorectum contains a marker which produces coloration with Schiff's reagent. This assay does not require detecting the disaccharide marker beta-D-Gal(1->3)-D-GalNAc, as required by the prior art. In fact, this disaccharide does not react with the Schiff's reagent.

It is an object of the present invention to provide a tool for the screening of asymptomatic persons for cancer of the large bowel and rectum.

It is a further object of the present invention to provide a screening test to detect neoplasms of the large bowel and rectum prior to development of a bleeding cancer.

It is yet a further object of the present invention to provide a screening test for colorectal cancer which does not give a high percentage of false positive and false negative results.

It is still yet a further object of the present invention to provide a screening test kit by means of which said test can be conducted outside of a hospital, medical

- 9 -

laboratory or clinic.

These and other objects and advantages of the invention will be seen from a reading of the specification as a whole.

Accordingly, the invention provides in one aspect a method for detecting the presence of precancer or cancer of the colon or rectum, which method comprises obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent and detecting precancer or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment.

Thus, the invention is a method for detecting the presence of neoplasia or cancer of the colon or rectum, which method consists essentially of obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent; and detecting neoplasia or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment.

For the purpose of clarification the process of the invention does not require treatment of the mucus sample with an enzyme for detecting the disaccharide marker beta-D-gal (1>3)-D-GaLNAC prior to treatment with Schiff's reagent.

The aforesaid coloration does not develop with basic fuchsin alone, although the Schiff's reagent itself is prepared from basic fuchsin (11).

The important advantage of testing rectal mucus, compared to lectin or antibody binding to histological sections of tumour tissue, is the easy accessibility of the material to be tested. Since the luminal surface of the colon is lined throughout its length with mucus, a viscoelastic gel composed of water, electrolytes, organic chemical substances, and large molecular weight glycoproteins (mucins), as well as sloughed cells and bacteria, which is movable along the bowel, it is suggested that rectal mucus contains mucus from the entire colon, i.e., the mucus secreted by a neoplastic tissue flows along the bowel into the rectum at which point it is sampled.

A mucus sample obtained by a physician or a trained

- 10 -

nurse from a screened subject is deposited on a suitable water-insoluble substrate or support, such as a pad or disc. Suitable support materials are, for example, glass microfibres Whatman GF/C, polymer fibres such as Biotrace RP, Metricel DM 450, Metricel VM-1, Sepraphore III, Versapore 450, or cellulose fibre such as Whatman 3MM. The support may or may not be pre-treated with an antioxidant such as BHT (butylated hydroxytoluene) or BHA (butylated hydroxyanisol).

Two procedures are preferably employed.

In procedure A, the mucus sample is deposited on a support as described hereinbelow, the mucus-carrying support is rinsed in potassium phosphate buffer, generally for about 10 minutes, then water, excess water removed and the support placed in Schiff's reagent for a short period of time, such as 1 minute, washed briefly with distilled water, and dried, for example in air or by pressing it between two stacks of filter paper or both. A positive reaction is scored when a purple-magenta colour appears on the filter in 20-25 minutes.

In procedure B, the mucus sample is deposited on a support already containing Schiff's reagent, as described hereinbelow. The support develops the purple-magenta colour within a short period of time, such as 30-60 seconds after the mucus specimen is deposited, if the marker is present.

The support, such as a pad, is, for procedure B, dipped in or otherwise treated with the solution of Schiff's reagent of appropriate strength to provide an effective, suitable amount retained on the pad to effect appropriate detection by colouration of the marker.

We have observed that the mucus smeared-pad, when exposed to air for prolonged periods of time, usually at least one hour, after treatment with Schiff's reagent, becomes uniformly coloured, due to the oxidation of the Schiff's reagent. Although a true positivity is readily distinguished from such background, such background can be further minimized by antioxidant pretreatment as hereinbefore described.

If a mucus specimen does not produce any coloration, it

- 11 -

is either because of the absence of the marker in the mucus, or because mucus was not collected by the gloved finger and therefore not deposited on the support. To distinguish between these two possibilities, the negative-testing mucus-treated support is treated with 0.5% periodic acid solution for 5 minutes, rinsed with water, stained with Schiff's reagent and rinsed again. When mucus lacking the marker is present, purple-magenta coloration appears; otherwise the support remains colourless, although some background coloration may develop.

During the practice of the process according to the invention, different shades of colour are sometimes observed. Such variations may reflect structural differences in the marker and could possibly correlate with clinical condition of the subject, e.g., chronic inflammation, ulcerative colitis, and the like.

It is known that the chemical properties of Schiff's reagent vary according to its method of preparation. Accordingly, a number of these variants were prepared and tested for use in the process of the present invention. The particulars of the various methods of preparation are listed hereinbelow. We have found that differences in colouration, sensitivity, specificity and oxidisability were obtained. While providing reasonably reproducible results, some of the Schiff's reagents listed below, for instance, were either overly sensitive, or poorly sensitive to the mucus specimen or gave a less than satisfactory background colouration. To obtain reproducible results with maximum sensitivity, the preferred Schiff's reagent No. 1 described below has been developed.

It is noted that commercial basic fuchsin (p-rosanilin) is often quite impure and Schiff's reagent is preferably prepared therefrom by purification before use. Sulphur dioxide, necessary for the preparation of the reagent, can be used as such in the gaseous form, or generated in situ from various precursors, such as NaHSO_3 , SOCl_2 , $\text{K}_2\text{S}_2\text{O}_5$, and $\text{Na}_2\text{S}_2\text{O}_5$, (12). We have found that the method of preparation of

- 12 -

the Schiff's reagent determines to some degree the reactivity, sensitivity, and stability of the reagent.

In a further aspect, the invention provides a screening kit comprising a container such as a package, carton, tube, box, roll, tape, or other capsule like object comprising a water-insoluble substrate capable of absorbing colorectal mucus and Schiff's reagent.

The support (substrate) may have been pre-treated with a solution of the Schiff's reagent to retain an active portion thereof; or the container may have each of the support and the Schiff's reagent separately packaged; or the Schiff's reagent may be generated before use from basic fuchsin deposited on the support.

In a further aspect, the invention provides a screening kit as hereinabove defined but incorporating packaged basic fuchsin instead of Schiff's reagent. The basic fuchsin is provided as a source of Schiff's reagent by subsequent reaction of sulphur dioxide with the basic fuchsin.

DESCRIPTION OF PREFERRED EMBODIMENTS

In order that the invention may be better understood preferred embodiments will now be described by way of example only, with reference to the accompanying drawing wherein the Figure shows a perspective view of an apparatus of use in the practice of the invention.

The Figure shows generally as 10, a frame assembly comprising a pair of rectangularly-shaped plates 12 and 14 (10cm x 10cm x 3mm) formed of Plexiglass® thermoplastic. Upper plate 12 is operably superimposed upon lower plate 14 in firm abutment together by means of a pair of opposed clips 16.

Plate 12 has a pair of circular apertures 18 (2 cm diameter). Retained between plates 12, 14 directly below apertures 18, as to be accessible therethrough, is a pair of disc supports 20, formed of Sepraphore III polymer film (2.5cm diameter).

- 13 -

In operation, a physician or nurse, for example, smears a mucus specimen onto the surface of each of pads 20, via apertures 18. Clips 16, are released and discs 20 processed as hereinbelow described, either in reference to Procedure A, or the subsequent results read directly in frame assembly 10 according to procedure B. Discs 20 are subsequently removed and discarded. Plates 12 and 14 are generally washed and re-used.

Two general procedures are hereinbelow described as a screening test for the early detection of colorectal neoplasia.

Procedure A:

A Sepraphore III support in the form of a disc, upon which a mucus specimen, obtained during the digital rectal examination, is smeared, is fastened in a frame formed by two square Plexiglass® plates, which may be conveniently handled in a physicians office. A suitable lubricant, such as glycerol, for the rectal examination is chosen from among those that do not react with Schiff's reagent. For processing, the following method has been found to be suitable, because it minimizes background coloration.

A support bearing a smeared-on mucus specimen is placed in 0.1M potassium phosphate buffer (pH 6.5-6.7) for 10 minutes, taken out and washed briefly with distilled water. Excess water is removed by placing the support on a cellulose filter with the side bearing the mucus smear in an up position. The support is then placed in Schiff's reagent denoted No.1 described hereinbelow, for 1 minute, taken out, washed briefly with distilled water, squeezed between two cellulose filters, and air dried. A positive reaction is scored when a purple-magenta colour appears on the filter within 20-25 minutes. Omitting the buffer wash leads to a decreased test specificity even if the sensitivity is maintained.

Stools deposited on the support together with the mucus cause an unwanted transformation in deposited mucus to take place during the storage before development, to result in a

- 14 -

positive test reading. To prevent this transformation from happening, a pretreatment of the mucus free support is carried out before use with 0.1% solution of an antioxidant, such as, for example, BHT in 95% ethanol, or BHA.

5 Two mucus samples are preferably obtained from each subject, one sample is used for testing and the other used for confirmatory purposes. It should be noted that usually unequal quantities of mucus are found deposited on the supports. The specimens are then treated with the Schiff's reagent, the results are recorded, and the specimens further
10 treated with periodic acid-Schiff's reagent to determine the quantity of mucus deposited. This procedure has shown that a weakly positive mucus-Schiff test result is to be expected if only a small amount of mucus is present on the support.
15 This has, thus, the same validity as a strongly positive result of an abundant mucus sample.

Procedure B: A suitable support is prepared as follows: A cellulose pad or disc (Whatman 3MM) is soaked in 0.1% solution of BHT in 95% ethanol and dried. Then it is soaked
20 in Schiff's reagent #1, and dried. This pre-treated support can be stored at -20 °C, or sealed to prevent contact with air. Alternatively, the support after deposition thereon of Schiff's reagent, for additional protection, may be again soaked in BHT solution and dried.

25 The specimen of mucus obtained on a gloved finger after digital sampling is smeared on this support. The specimen is positive for the marker if the coloration develops within approximately up to one minute after the specimen was deposited on the support. Later colour development does not
30 represent positivity.

Since the treatment with phosphate buffer solution is omitted in procedure B, the specificity of procedure B is lower than that of procedure A (from 80-90% to 60-70%). Since the sensitivity remains close to 100%, the negative
35 results represent the disease-free individuals. Positive results can be reexamined by procedure A to increase specificity of the testing.

- 15 -

A number of Schiff's reagents of use in the invention are prepared as follows.

1. Basic fuchsin (0.2 g) is dissolved in hot water (100 mL), boiled for 5 minutes, filtered and cooled to room temperature. Sodium bisulfite (1.17 g) and 1N hydrochloric acid (17 mL) are added sequentially to the filtrate and the solution allowed to stand in a dark place at room temp. for 4 days. Decolorizing charcoal (0.15 g) is added, mixed well, and filtered off. The resulting colourless or slightly yellow solution is stable for suitable prolonged periods of time. The reagent is stored at +4°C in a refrigerator.

2. Basic fuchsin (1.0 g) is dissolved in hot water (200 mL), boiled for 5 minutes, filtered and cooled to room temperature. Then sodium metabisulfite (1.0 g) and 1N hydrochloric acid (20 mL) are added sequentially to the filtrate and the solution allowed to stand in a dark place at room temp. for 4 days. Charcoal (0.3 g) is added, mixed well, and filtered off.

3. Basic fuchsin (1.0 g) is dissolved in hot water (200 mL), boiled for 5 minutes, filtered and cooled to room temperature. Then potassium metabisulfite (1.0 g) and 1N hydrochloric acid (20 mL) are added sequentially to the filtrate and the solution allowed to stand in a dark place at room temp. for 4 days. Charcoal (0.3 g) is added, mixed well, and filtered off.

4. Basic fuchsin (1.0 g) is dissolved in hot water (200 mL), boiled for 5 minutes, filtered and cooled to room temperature. Then potassium metabisulfite (1.0 g) and 1N hydrochloric acid (25 mL) are added sequentially to the filtrate and the solution allowed to stand in a dark place at room temp. overnight. If the solution is still coloured, two drops of 6N hydrochloric acid are added, stored in a dark place for 48 hours. Then charcoal is added, mixed well, and filtered off.

5. Basic fuchsin (0.2 g) is dissolved in hot water (100 mL), boiled for 5 minutes, filtered and cooled to room temperature. After thionyl chloride (7-8 mL) is added to the

- 16 -

filtrate, the solution is allowed to stand in a dark place at room temp. for 24 hours. Charcoal is added to decolorize the solution, mixed well, and filtered off.

5 6. Basic fuchsin (1.0 g) is dissolved in 0.25M hydrochloric acid (100 mL) and sodium bisulfite (10.0 g) is added. The solution has pH=3.

10 7. Basic fuchsin (0.12 g) is dissolved in hot water (200 mL), boiled for 5 minutes, filtered and cooled to room temperature. Then glacial acetic acid (11.40 mL) and sodium bisulfite (1.0 g) are added sequentially to the filtrate. The solution is slightly pink in colour.

15 8. Commercial Schiff's reagent (Fischer) pH=1 is adjusted to pH=3 by the addition of aqueous sodium hydroxide (0.2N). The resultant pink solution is decolorized with sodium bisulfite.

20 9. Basic fuchsin (1.0 g) is dissolved in hot water (200 mL), boiled for 5 minutes, filtered and cooled to room temperature. Then sodium bisulfite (1.0 g) and 1N hydrochloric acid (25 mL) are added sequentially to the filtrate and the solution allowed to stand in a dark place at room temp. for 48 hours. Charcoal (0.3 g) is added, mixed well, and filtered off.

25 10. Basic fuchsin (1.0 g) is dissolved in hot water (200 mL), boiled for 5 minutes, filtered and cooled to room temperature. Then sodium bisulfite (2.0 g) and 1N hydrochloric acid (25 mL) are added sequentially to the filtrate and the solution allowed to stand in a dark place at room temp. for 48 hours. Charcoal (0.3 g) is added, mixed well, and filtered off.

30 11. To commercial Schiff's reagents (Sigma) pH=1.5 (100 mL) is added glacial acetic acid (4.5 mL).

EXAMPLES

Example 1

35 In all investigated groups, using modified procedure A, clinically diagnosed cancers were detected with high

- 17 -

sensitivity, minimum 92%. In one study, out of 25 cancer patients, 23 gave a positive result in the mucus-Schiff test; of 30 large adenomatous polyps, 24 gave positives; of 76 small polyps, 43 gave positives.

5 It has been shown by the results in this group that colonic inflammatory conditions such as ulcerative colitis, diverticulitis, Crohn's disease, acute and chronic inflammation often give positive results (Table). Since at least some of these conditions have been recognized as risk factors for colorectal cancer, these results show that the test recognizes either susceptible individuals or an early stage of neoplastic development. The latter argument applies to polyps as well.

15 Example 2

A study was designed to compare patients visiting the endoscopy unit with patients visiting physicians' (gastroenterologists') offices for non-malignant disease. The visits were due to unspecified complaints. The latter group consisted of 45 patients and the former of 39 patients. Two specimens were prepared from each patient. Modified procedure A was used. The two main differences between the groups were: (a) the endoscopy suite group received colonic lavage beforehand to free the colon of faeces, and was on a liquid diet for 24 hours prior to the mucus collection (and colonoscopy), while (b) the other group did not receive lavage and had no diet restrictions.

25 The results show positivity of 27% (95% confidence interval: 15.4-42.6), 12/45 among patients in physicians' offices and 33% (95% confidence interval: 19.3-48.4), 13/39 among patients in the endoscopy suite. This study thus shows that the presence of faeces in specimens, and normal food intake, do not determine the percentage of the positivity. The positivity is consequently due to a condition other than colorectal cancer.

- 18 -

Example 3

The specificity measured in clinical control populations is very imprecise in patients, who at the moment of the test, have no clinically detected neoplasms but have some other unspecified ailments, which may well predispose to cancer formation in the future. Thus, a study was designed to determine the number of positives in a cohort of 47 young adult volunteers unlikely to suffer of any intestinal ailment or neoplastic condition. All individuals were between 18 to 35 years of age and feeling completely healthy. This investigation was expected to provide an estimate of specificity of the investigated screening tool. Four specimens were collected from each individual.

The results described in Examples 2 and 3 suggest that some individuals may have a condition increasing the risk of neoplasia. The false positive rate among healthy young volunteers (not patients) was found to be low (10.6%). A segment of inflamed bowel may, in fact, be transformed into a preneoplastic condition, and this perhaps is detected by the test.

The high sensitivity and relatively high specificity of the test for neoplasms may reduce the number of patients undergoing colonoscopy because they have rectal bleeding, unexplained iron-deficiency anemia, or a first-degree relative with a tumour.

The Table shows the results of Schiff's reagent analysis of mucus from a group of patients with colorectal cancer and putative precancerous conditions at the endoscopy unit at the Wellesley Hospital, Toronto, Ontario, Canada, who agreed to submit themselves to the mucus testing.

The following notes provide a better understanding of the Table:

- (a) The two cancer patients listed as negative had an ambiguous reaction due to very small amount of mucus deposited on pads. They underwent

- 19 -

surgery before the test could be repeated;

(b) The positivity/negativity of polyps reflects the well-known observation that some polyps are cancer precursors whereas some are not;

(c) Laser treatment probably inhibits mucus production by tumour, especially if the laser coagulation is extensive;

(d) Positivity/negativity of the test in previously removed carcinomas may reflect the completeness of the cancer removal;

(e) Inflammatory conditions are considered a risk factor for colonic cancer. The positivity in the test may reflect how far an inflammation has progressed to an early stage of cancer development;

(f) *Percentage of groups with less than 10 subjects are not calculated; and

(g) **CI, Confidence Interval of percentage positive.

Although this disclosure has described and illustrated certain preferred embodiments of the invention, it is to be understood that the invention is not restricted to those particular embodiments. Rather, the invention includes all embodiments which are functional or mechanical equivalents of the specific embodiments and features that have been described and illustrated.

- 20 -

TABLE

| Diagnosis | total # of case | positive | | 95 % CI** | negative | |
|---|--------------------|----------|----|-----------|----------|----|
| | | # | % | | # | % |
| Cancer | 25 | 23 | 92 | 72.5-98.6 | 2 | 8 |
| Polyp adenomatous (< 1 cm in diameter) | 76 | 43 | 57 | 45.1-68.1 | 33 | 43 |
| Polyp adenomatous (< 1 cm in diameter; removed endoscopically 2-8 weeks prior to mucus collection) | 5 | 3 | | | 2 | |
| Polyp adenomatous (< 2 cm in diameter) | 30 | 24 | 80 | 59.7-91.6 | 6 | 20 |
| Carcinoma (laser treated) | 6 | 1 | | | 5 | |
| Carcinoma (removed 2-8 weeks before mucus collection) | 7 | 5 | | | 2 | |
| Ulcerative colitis | 6 | 4 | | | 2 | |
| Crohn's disease | 7 | 3 | | | 4 | |
| Diverticulitis | 1 | 1 | | | | |
| Acute inflammation | 2 | 2 | | | | |
| Chronic inflammation | 1 | 1 | | | | |
| Acute colitis | 1 | | | | 1 | |
| TOTAL | <u>167</u> | | | | | |

- 21 -

We claim:

1. A method for detecting the presence of neoplasia or cancer of the colon or rectum, which method comprises obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent; and detecting neoplasia or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment.

2. A method for detecting the presence of neoplasia or cancer of the colon or rectum, which method consists essentially of obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent; and detecting neoplasia or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment.

3. A method for detecting the presence of neoplasia or cancer of the colon or rectum, which method comprises obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent, without a step of adding an enzyme for detecting the disaacharide marker beta-D-Gal (1->3)-D-GalNAc, ; and detecting neoplasia or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment.

4. A method for detecting the presence of neoplasia or cancer of the colon or rectum, which method consists essentially of obtaining a sample of colorectal mucus from the rectum of a patient; treating said sample with Schiff's reagent, without a step of adding an enzyme for detecting the disaccharide marker beta-D-Gal (1->3)-D-GalNAc; and detecting neoplasia or cancer of the colon or rectum based upon the coloration produced in said sample by said treatment.

- 22 -

5. A process as defined in any one of claims 1, 2, 3 or 4 wherein said mucus is absorbed onto a water-insoluble substrate.

5 6. A process as defined in claim 5 wherein said water-insoluble substrate has been pre-treated with an anti-oxidant.

10 7. A screening kit for detecting the presence of neoplasia or cancer of the colon or rectum as defined in any one of claims 1, 2, 3 or 4, which kit comprises a container comprising a water-insoluble support capable of absorbing said colorectal mucus, and Schiff's reagent.

15 8. A kit as defined in Claim 7 wherein said support has been pre-treated with said Schiff's reagent.

20 9. A kit as defined in Claim 7 wherein said support and said Schiff's reagent are separately packaged.

25 10. A screening kit for detecting the presence of neoplasia or cancer of the colon or rectum as claimed in any one of Claims 1, 2, 3 or 4 which kit comprises a container comprising a water-insoluble support capable of absorbing said colorectal mucus, and basic fuchsin for subsequent generation of Schiff's reagent.

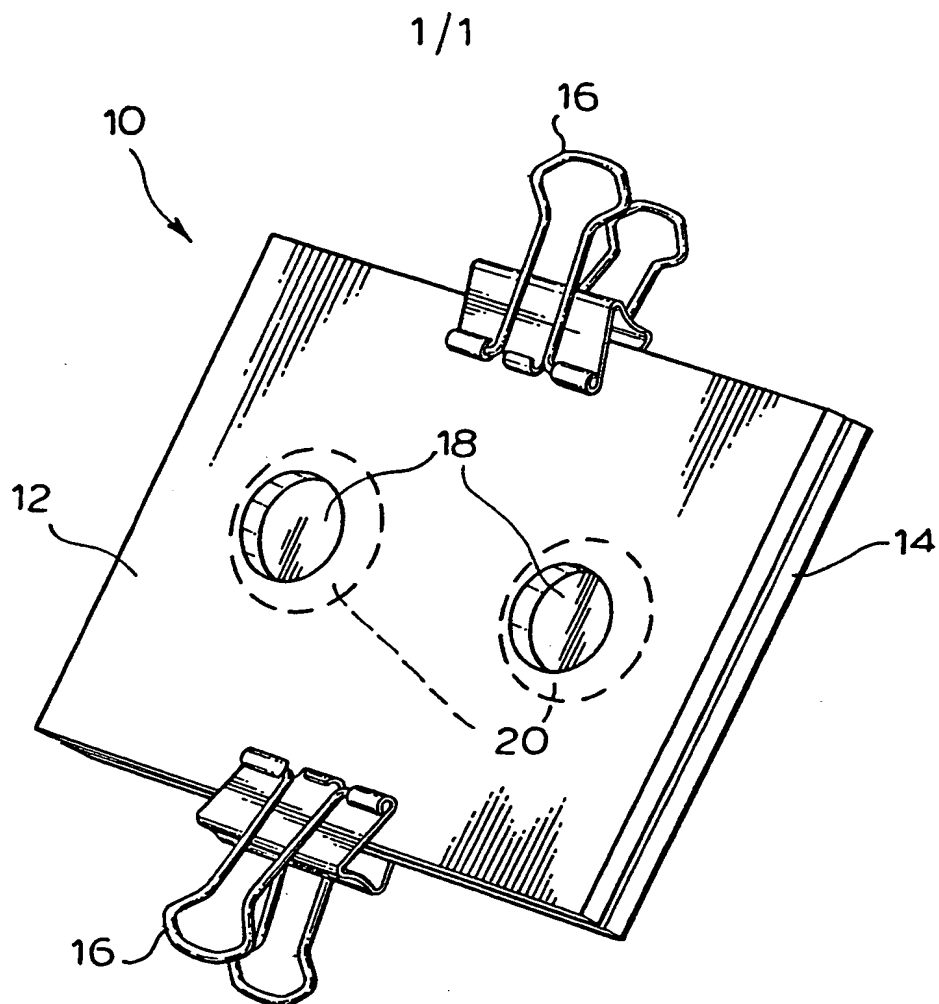


FIG.1.

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 94/00646

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/52 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | WO,A,91 09309 (SHAMSUDDIN, ABULKALAM) 27 June 1991 see the whole document | 1,2,5, 7-10 |
| A | see the whole document --- | 3,4,6 |
| A | WO,A,88 00702 (SHAMSUDDIN, ABULKALAM; ELSAYED ALAA-EL-DEEN; JOCKLE, GLENN A.) 28 January 1988 & US,A,4 857 457 (SHAMSUDDIN ET AL.) 15 August 1989 cited in the application see the whole document --- | 1-10 |
| -/-- | | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

29 March 1995

Date of mailing of the international search report

05.04.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Döpfer, K-P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 94/00646

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | <p>CANCER BIOTHERAPY, vol.8, no.1, 1993, NEW YORK,NY,US pages 49 - 55 KOSAKU SAKAMOTO ET AL. 'Evaluation of a New Test for Colorectal Neoplasms: A Prospective Study of Asymptomatic Population' see page 50, right column, line 38 - page 51, left column, line 14 see abstract</p> <p style="text-align: center;">---</p> | 1-10 |
| A | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol.79, no.3, March 1982, WASHINGTON US pages 2051 - 2055 C. RICHARD BOLAND ET AL. 'Alterations in human colonic mucin occurring with cellular differentiation and malignant transformation' cited in the application</p> <p style="text-align: center;">-----</p> | |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 94/00646

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO-A-9109309 | 27-06-91 | US-A- 5162202 | 10-11-92 |
| | | AU-A- 6974691 | 18-07-91 |
| | | JP-A- 3191798 | 21-08-91 |
| | | US-A- 5348860 | 20-09-94 |
| ----- | | | |
| WO-A-8800702 | 28-01-88 | US-A- 4857457 | 15-08-89 |
| | | AU-B- 615419 | 03-10-91 |
| | | AU-A- 7757587 | 10-02-88 |
| | | EP-A- 0293395 | 07-12-88 |
| ----- | | | |
| US-A-4857457 | 15-08-89 | AU-B- 615419 | 03-10-91 |
| | | AU-A- 7757587 | 10-02-88 |
| | | EP-A- 0293395 | 07-12-88 |
| | | WO-A- 8800702 | 28-01-88 |
| ----- | | | |